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TITLE: DETERMINATION OF PARAMETERS FOR DEVELOPMENT OF A PHYSIOLOGICALLY BASED MODEL FOR THE TOXICOKINETICS OF C(±)P(±)-SOMAN

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13. ABSTRACT *(Maximum 200 words)*

Toxicant-specific parameters needed for development of a physiologically based model for the toxicokinetics of C(±)P(-)-soman in guinea pig were determined in blood, and in homogenates of brain, liver, kidney, lung, and a skeletal muscle (gastrocnemius et soleus). Tissue/blood partition coefficients for the diastereoisomers of C(±)P(±)-soman were obtained from the partition coefficients determined for blood/air and tissue homogenate/air by gas chromatographic analysis in the air phase. Elimination of C(±)P(±)-soman by covalent binding was blocked by pretreatment with the very labile crotyl sarin (2-butenyl methylphosphonofluoridate). Enzymatic hydrolysis was stopped by lowering the pH. Relatively high concentrations of sites for covalent binding of C(±)P(-)-^{14}C-soman were found in liver and kidney homogenates, whereas these concentrations were low in the target organs, i.e., brain and muscle. Only a fraction of the available binding sites reacts rapidly with C(±)P(-)-soman. Indications were obtained for contribution of C(±)P(+)-soman to binding in liver and kidney homogenates. The half-life times of hydrolysis for C(-)P(-)-soman in plasma and in tissue homogenates were similar to the values obtained previously for C(+)P(-)-soman hydrolysis. In addition, the cardiac output and tissue blood flows were determined in anaesthetized, atropinized and artificially ventilated guinea pigs before as well as 10 min after intravenous administration of 2 and 6 LD_{50} C(±)P(±)-soman. Neither the cardiac output nor the blood flow distribution was significantly affected by the intoxication.

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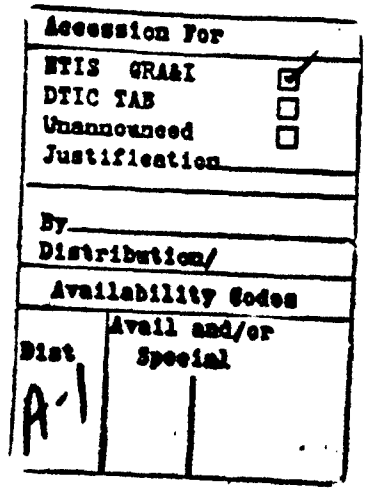

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SUMMARY

Physiologically based models provide a basis for extrapolation of toxicokinetic data from laboratory animals to man. Our previous studies provide data for validation of a model describing the toxicokinetics of the toxic stereoisomers of C(±)P(±)-soman and some toxicant-specific parameters needed for development of the model. The present report completes this set of toxicant-specific parameters for the guinea pig, i.e., tissue/blood partition coefficients, concentrations of covalent binding sites, rate constants for covalent binding by C(±)P(-)-^{14}C-soman and half-life times for hydrolysis of C(-)P(-)-soman. Parameters were determined in blood, and in homogenates of brain, liver, kidney, lung, and a skeletal muscle (gastrocnemius et soleus). In addition, the cardiac output and tissue blood flows were determined under circumstances prevailing during measurement of the relevant toxicokinetic data.

The tissue/blood partition coefficients for the diastereoisomers of C(±)P(±)-soman were similar. These coefficients were obtained from the partition coefficients determined for blood/air and tissue homogenate/air by gas chromatographic analysis in the air phase by using a head-space injection technique. Elimination of C(±)P(±)-soman by covalent binding in blood and tissue homogenates was effectively blocked by pretreatment with crotyl sarin (2-butenyl methylphosphonofluoridate), which rapidly reacts with covalent binding sites, but is also rapidly dealkylated to a nonvolatile methylphosphonofluoridic acid. Enzymatic hydrolysis was stopped by lowering the pH to 3.3. Relatively high concentrations of sites for covalent binding of C(±)P(-)-^{14}C-soman were found in liver and kidney homogenates, whereas these concentrations were low in the target organs, i.e., brain and muscle. Only a fraction of the available covalent binding sites in guinea pig blood and tissue homogenates reacts rapidly with C(±)P(-)-soman. Indications were obtained for contribution of C(±)P(+)-soman to covalent binding in guinea pig liver and kidney homogenate when treated with an equimolar mixture of C(±)P(-)-^{14}C-soman and C(±)P(+)-soman. The method used was not adequate for evaluation of the contribution of C(±)P(+)-soman to covalent binding in homogenates of the other tissues or in blood. The half-life times of hydrolysis for C(-)P(-)-soman in guinea pig plasma and in various tissue homogenates were similar to the values obtained in a previous study for C(+)P(-)-soman hydrolysis. The hydrolytic activities for C(±)P(-)-soman in target tissues (brain, muscle) of the guinea pig are lower than in most of the tissues participating in central elimination, similarly to the binding capacities for C(±)P(-)-soman in these tissues.

Cardiac output and blood flow distributions in various tissues were determined in anaesthetized, atropinized and artificially ventilated guinea pigs before as well as 10 min after intravenous administration of 2 and 6 LD_{50} C(±)P(±)-soman. Neither the cardiac output nor the blood flow distribution was affected by the intoxication.

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I. INTRODUCTION

During the last couple of years, we and others have performed a number of studies on the toxicokinetics of 1,2,2-trimethylpropyl methylphosphonofluoridate [C(±)P(±)-soman, 1-11] and related organophosphates (12-15). These studies afforded a better insight into the persistence of the agents in the body, from which improvements of therapy and pretreatment of organophosphate intoxication could be conceived (3,4,16).

In our studies performed in rat, guinea pig, and marmoset, the biochemical and toxicological implications of the chirality of C(±)P(±)-soman have been taken into account. The nerve agent consists of four stereoisomers denoted as C(+)P(+)-, C(+)P(-)-, C(-)P(+)-, and C(-)P(-)-soman, in which C stands for the asymmetric carbon in the pinacolyl moiety and P for the asymmetric phosphorus atom. Only the C(±)P(-)-isomers are highly toxic (17). Our toxicokinetic studies showed that these isomers are much more persistent in the three species than the relatively nontoxic C(±)P(+)-isomers. It was estimated from the data obtained that toxicologically significant levels of C(±)P(-)-soman persist at intravenous (i.v.) doses of 6 LD_{50} for approximately 5, 2, and 1 h in the rat, guinea pig, and marmoset, respectively (2-4). Such levels persist in the guinea pig for approximately 4 h after subcutaneous (s.c.) administration of 6 LD_{50} of the agent (5), and for approximately 0.5 h after i.v. infusion with 0.8 LD_{50} (8) and after respiratory exposure to 0.8 LCt_{50} (7) of the agent. We further concluded from these studies that the guinea pig is a better model for a primate than the rat from the point of view of toxicokinetics. Additional investigations of the elimination pathways indicated that the C(±)P(-)-isomers are preferentially eliminated by covalent binding, for instance, to carboxylesterases, while elimination of the C(±)P(+)-isomers mainly proceeds by way of enzymatic hydrolysis (3,4).

The relatively high persistence of the toxic C(±)P(-)-isomers suggests that scavengers applied in a therapeutic situation should support the effects of the conventional treatment of C(±)P(±)-soman intoxication with atropine and oxime. Furthermore, computer simulations showed that the efficacy of the pretreatment of C(±)P(±)-soman intoxication, e.g., with pyridostigmine, may be limited in particular by the persistence of C(±)P(-)-isomers in the terminal elimination phase (16). Also for this reason, the use of additional antidotes that accelerate the terminal elimination, such as scavengers, may substantially improve the efficacy of the pretreatment.

Although the results of the toxicokinetic studies already lead to valuable suggestions with respect to therapy and pretreatment of C(±)P(±)-soman intoxication, these conclusions would be more generally applicable if the toxicokinetics can be described in a physiologically based model (18-21). These models represent the mammalian system in terms of specific tissues or groups of tissues, all connected by arterial and venous blood flow pathways. The models

use physiological parameters, such as tissue volumes and blood flow rates, and parameters specific for the chemical agent under investigation, such as tissue/blood partition coefficients and metabolic parameters. The models consist of a set of mass-balance differential equations for the various tissues and groups of tissues Based on these differential equations, time-dependent toxicokinetic data can be simulated. The coherent relationship among anatomical and physiological characteristics of different species provides the basis for cross-species scaling of toxicokinetic data described in such a model and extrapolation eventually to man. Maxwell et al. (11) described a first physiologically based model for C(±)P(±)-soman and, recently, Gearhart et al. (22) developed a similar model for diisopropyl phosphorofluoridate (DFP), a toxic agent closely related to C(±)P(±)-soman.

In addition to the physiological and toxicant-specific parameters, which are needed for development of a physiologically based model, kinetic data should be available on which the developed model is validated. A direct validation is carried out by comparison of the time course of blood concentrations predicted on the basis of the model with these data measured for the toxicant. The models for organophosphates described so far, however, were validated by using the time course of acetylcholinesterase (AChE) inhibition, the target for the toxic effects of the organophosphates. Probably, the limited number of data available for blood levels of the agents was insufficient for proper testing of the models.

Our studies on biochemical and toxicological implications of chirality in C(±)P(±)-soman clearly indicate that discrimination between the toxic C(±)P(-)-isomers and the nontoxic C(±)P(+)-isomers is essential when modelling the toxicokinetics of the agent. These pairs of isomers differ not only with respect to toxicity (17), but also with respect to elimination (2-4) as mentioned before. Therefore, it is concluded that a physiologically based model for the toxicokinetics of soman should be based on toxicant-specific parameters for the toxic C(±)P(-)-isomers and should be validated on levels measured for these isomers.

The latter data are provided by our toxicokinetic studies, i.e., time courses of blood levels for C(±)P(-)-soman following i.v. and s.c. bolus administration, as well as during and after i.v. infusion of C(±)P(±)-soman and respiratory exposure to the agent. Rate constants for absorption phases after administration (s.c., i.v. infusion and respiratory exposure) were also determined. Moreover, data are available on the major elimination pathway for the toxic isomers, i.e., quantities of C(±)P(±)-soman covalently bound 1 h after i.v. administration of the agent.

In the course of our studies on the toxicokinetics of C(±)P(±)-soman we obtained some toxicant-specific parameters, such as totally available binding sites for C(+)P(-)-soman and half-life times for hydrolysis of C(+)P(-)-soman in plasma and a number of tissues. In this report we describe the determination of additional toxicant-

specific parameters of C(±)P(-)-soman as well as physiological parameters of C(±)P(±)-soman-intoxicated animals that were needed for modelling. Since our toxicokinetic studies indicate that the guinea pig is a better model for primates than the rat, this species should be the species of choice for toxicokinetic modelling.

The results will be used for development of a physiologically based model for the toxicokinetics of C(±)P(-)-soman and for subsequent validation of the model from comparison of predicted values with our experimental data (2-5), in a joint effort with the U.S. Army Medical Research Institute of Chemical Defense.

Physiologically based models consist of both organ-specific and lumped compartments. Organ-specific compartments are directly involved in the acute toxic effect of the agent or have a major influence on the toxicokinetics. The lumped compartments are groups of tissues that have common characteristics and can jointly be described without significant loss of critical information. In this study we determined toxicant-specific parameters in blood, brain, liver, kidney and lung as organ-specific compartments. Furthermore, these parameters were determined in liver and skeletal muscle, which are considered as representative tissues for richly perfused and slowly perfused tissues, respectively.

The following toxicant-specific parameters were determined.

(i) *Parameters for partitioning of the C(±)P(±)-soman stereoisomers between blood and tissues*

These parameters, which are not yet available for guinea pig blood and tissues, will be obtained by equilibration of C(±)P(±)-soman in a liquid/gas system, e.g., blood/air and tissue homogenate/air, and by subsequent analysis of C(±)P(±)-soman in the head space by means of gas chromatography (23,24). The parameters will be identical for enantiomers of a chiral compound, but may be different for the two enantiomeric pairs of C(±)P(±)-soman, i.e., [C(+)P(+)- + C(-)P(-)-soman] and [C(+)P(-)- + C(-)P(+)-soman]. Our experimental setup allowed the simultaneous determination of the partition coefficients for these two pairs of enantiomers.

(ii) *Parameters for covalent binding*

From a toxicological point of view, the most important binding process of C(±)P(±)-soman is its reaction with AChE which induces the intoxication by the agent. The molar quantities of AChE and of other cholinesterases, however, represent only a very small percentage of the total binding sites in mammals (3,4), which can be neglected from a point of view of C(±)P(±)-soman elimination. Consequently, discrimination between binding to cholinesterases and binding to other sites will not be necessary for a description of the elimination of C(±)P(±)-soman.

In the course of our previous investigations (3,4) we already collected data on the binding capacity for C(+)P(-)-soman in various tissues and data for the occupation of the binding sites by C(±)P(±)-soman 1 h after i.v. administration of 2 and 6 LD_{50} C(±)P(±)-soman.

In the present study the overall binding capacities for the toxic C(±)P(-)-soman were determined in the various tissues. Apart from parameters for toxicokinetic modelling, the concentrations of binding sites obtained were needed for determination of rate constants for the binding reactions. Probably, various proteins may serve as binding sites, which may have widely varying reactivities for C(±)P(-)-soman binding. Therefore, we determined overall rate constants for binding in blood and in homogenates of the tissues of choice. Since we observed in previous studies (4) that covalent binding sites are occupied mainly, but not exclusively, by C(±)P(-)-soman after i.v. administration of C(±)P(±)-soman to guinea pigs, determinations of the rate constants for binding were carried out in the presence of C(±)P(+)-soman in order to account for the possible contribution of these isomers.

(iii) *Parameters for enzymatic hydrolysis*
Parameters have already been obtained for enzymatically catalyzed hydrolysis of C(+)P(-)-soman from hydrolysis of the isomer in plasma as well as in 25% homogenate of various tissues and 10% muscle homogenate (3). Similar experiments were now carried out for the other toxic isomer. Since our toxicokinetic studies indicated that hydrolysis of C(±)P(-)-soman contributes to its elimination to a much larger extent in the terminal phase than in the initial phase, we chose an initial C(-)P(-)-soman concentration of ca. 40 ng/ml, i.e., corresponding with the concentration present in blood after the initial rapid concentration decay following administration of 2-6 LD_{50} C(±)P(±)-soman. The reactions were followed by gas chromatographic analysis of the residual concentration of the isomer.

(iv) *Parameters for tissue blood flow and cardiac output*
The aim of our toxicokinetic studies (2-5) is to provide more insight into the persistence of C(±)P(±)-soman in the body, in order to improve therapy and pretreatment of intoxication by the agent. Therefore, we performed these studies at lethal doses of C(±)P(±)-soman, i.e., 2 and 6 LD_{50}, in atropinized animals. Since data obtained from these studies will be used to validate the physiologically based model to be developed, the physiological parameters of interest should be determined under conditions similar to those prevailing when the toxicokinetic data were obtained. Within this context it should be noted that C(±)P(±)-soman is rapidly distributed over the body after administration (2,3,5). In accordance with this rapid distribution, there is a rapid onset of the effects of the intoxication. Consequently, changes in cardiac output and tissue blood flows induced by C(±)P(±)-soman intoxication at high dose will occur shortly after administration of the agent. For this reason, we determined the cardiac output and tissue blood flows in anaesthetized, atropinized and artificially ventilated animals in order to obtain these physiological parameters for the conditions prevailing in our toxicokinetic studies shortly before C(±)P(±)-soman administration. In addition, these physiological parameters were determined in anaesthetized, atropinized and artificially ventilated animals 10 min after i.v. administration of 2 and 6 LD_{50} C(±)P(±)-soman, in order to evaluate changes in the parameters due to

C(±)P(±)-soman intoxication. These determinations were carried out by using radioactively labelled microspheres as reported by Peeters et al. (25).

II. MATERIALS AND METHODS

II.1. Materials

C(±)P(±)-1,2,2-Trimethylpropyl methylphosphonofluoridate (soman), 2,2-dimethylpropyl methylphosphonofluoridate (neopentyl sarin) and 2-butenyl methylphosphonofluoridate (crotyl sarin) were prepared at TNO Prins Maurits Laboratory from reaction of the appropriate alcohol with methylphosphonic difluoride and methylphosphonic dichloride according to the procedure of Bryant et al. (26). The compounds were distilled over a Spaltrohr column until a purity ≥ 99% by gas chromatography was obtained. C(±)P(±)-1,2,2-Trimethylpropyl ^{14}C-methylphosphonofluoridate [C(±)P(±)-^{14}C-soman] was a gift of the Centre d'Études du Bouchet (Vert le Petit, France) and had a specific activity of 1.7 TBq/mole. The internal standard C(±)P(+)-[U-^{2}H]1,2,2,-trimethylpropyl methylphosphonofluoridate [C(±)P(+)-D_{13}-soman], C(±)P(+)-soman, C(±)P(-)-^{14}C-soman and C(-)P(-)-soman, were obtained as described previously (2,27,28).

Isopropanol (Brocacef, Rijswijk, The Netherlands) was distilled over a column packed with Dixon rings (plate number 80; NGW, Wertheim, West Germany). The following products were obtained commercially and were used without further purification: ethyl acetate (Merck, Darmstadt, Germany, zur Rückstandanalyse), saponin (BDH, Poole, UK), aluminium sulfate.16 H_2O (BDH Analar, ≥ 98%), sodium bicarbonate (Lamens en Indemans, 's-Hertogenbosch, The Netherlands, > 99.5%), acetic acid (Lamens en Indemans, > 99%), sodium acetate (Merck, zur Analyse, > 99.5%), atropine sulfate (Brocades Stheeman, Haarlem, The Netherlands), sodium barbital (Brocades Stheeman), halothane (Trofield, Zug, Switzerland), heparin (5000 I.U./ml, Kabi Vitrum, Stockholm, Sweden), fentanyl and fluanisone (Hypnorm^{R}; Duphar, Amsterdam, The Netherlands), pentobarbital sodium (Nembutal^{R}; Sanofi, Maassluis, The Netherlands), toluene (Lamens and Pleuger, 's-Hertogenbosch, The Netherlands, > 99.3%), sulfuric acid (Merck, zur Analyse), isobutanol (UCB, Brussels, Belgium, pour analyse), Hionic-Fluor (Packard Instruments B.V., Groningen, The Netherlands), Soluene-350 (Packard Instruments B.V.), ^{14}C-hexadecane (specific acitivity 340 kBq/mmol, Amersham, Houten, The Netherlands), and hydrogen peroxide (Merck, 30%, zur Analyse). Compressed gases for inhalation anaesthesia, i.e., oxygen for medical purposes and nitrous oxide, were obtained from Hoek-Loos (Amsterdam, The Netherlands).

NEN-TRAC microspheres (mean size 15.5 ± 0.1 μm) labelled with ^{141}Ce, specific activity 147.6 MBq/g, and with ^{103}Ru, specific activity 183.1 MBq/g, were purchased from DuPont (Boston, MA). Since the preparations contained $3.66*10^{5}$ microspheres/mg, the specific activities correspond with 24 dpm/microsphere for the ^{141}Ce-labelled product and 30 dpm/microsphere for the ^{103}Ru-labelled product. The microspheres were obtained as suspensions in 10% dextran with 0.01% Tween 80. These suspensions were diluted ca. 12 times with sterile saline. The microsphere suspensions were stored at 2-5 °C.

SepPak C_{18} columns were procured from Millipore (Waters Associates, Milford, MA).

II.2. Animals

Male albino outbred guinea pigs of the Dunkin-Hartley type were purchased from Charles River (Sultzfeld, Germany). The animals were allowed to eat and drink *ad libitum*. They were allowed to acclimatize to their new environment for at least 1 week before they were used in any experiment.

II.3. Collection of tissues used in the determination of partition coefficents and metabolic parameters

The animals were anaesthetized with 2.5% halothane in N_2O/oxygen (62:38) using a Fluotec 3 Continuous Flow Vaporizer (BOC Health Care, Ohmeda, Steeton, UK). The flow rates of oxygen and N_2O were determined with flowmeters (Rota, Oeflingen, Germany) and were adjusted with high precision flow regulators. Blood was obtained by heart puncture with heparinized syringes. Plasma was prepared by centrifugation for 20 min at 1200 g.
After collection of blood from the animals, tissues of interest were removed. For the biochemical experiments, homogenates were made 25% (w/w) for brain, liver, and kidney, 15% for lung, and 10% for gastrocnemius et soleus muscles, in 0.01 M veronal buffer containing sodium chloride (9 g/l), pH 7.5, with a Polytron PT 10S homogenizator. For the determination of the partition coefficients, all homogenates were 25% (w/v) in 0.9% sodium chloride in water (saline).
Plasma and homogenates were pooled from six guinea pigs.

II.4. *In vitro* determination of tissue/blood partition coefficients

Partition coefficients were determined according to the procedure described by Sato and Nakajima (23) and by Gargas et al. (24). All equilibration vials (volume, 9.28 ml; Chrompack, Middelburg, The Netherlands) used were silanized. Initially, enzymes in blood diluted with saline (1:3, v/v) and in the tissue homogenates were inactivated by treatment for 30 min at 90 °C or by acidification with 2 M acetate buffer, pH 3.3, (25 µl/ml of blood or homogenate). In the final experiments, inactivation was achieved by acidification with 2 M acetate buffer, pH 3.3, (25 µl/ml of blood or homogenate) after which 2 µl of a crotyl sarin solution (5 mg/ml isopropanol) was added per milliliter of blood or homogenate or, in case of liver and kidney homogenate, 1 µl of the neat compound was added per milliliter. After incubation for 5 min at 37 °C, 1 ml of treated homogenate, diluted blood or saline was pipetted into an equilibration vial to which 1 µl of a solution of 6.65 mg C(±)P(±)-soman/ml isopropanol was added. The same volume of this C(±)P(±)-soman solution was added to the reference vials which contained 0.75 ml saline or were empty vials in case of the determination of the saline/air partition coefficient. After equilibration for 2 h at 37 °C, the concentrations of

[C(+)P(+)- + C(-)P(-)-soman] and of [C(+)P(-)- + C(-)P(+)-soman] in the air phase were analyzed with a Carlo Erba HRGC Series 4160 gas chromatograph equipped with a Carlo Erba HS 250 head-space sampler. The volume of the gas sample loop was 1 ml. The two pairs of soman enantiomers were separated on a CPSil 8 CB fused silica column (length, 50 m; i.d., 0.32 mm; film thickness, 1.2 μm; Chrompack, The Netherlands). After injection, the column was held for 2 min at 80 °C, programmed to 130 °C at 5 °C/min, and subsequently held at 130 °C for 4 min. Carrier gas helium was used at a flow of 3.75 ml/min, and flows of air, hydrogen and make-up gas helium through the alkali flame detector were 326, 41 and 13 ml/min, respectively. The injector was held at 150 °C, the detector block at 300 °C. Under these conditions the retention times for the two pairs of soman enantiomers were 12-13 min. The determinations were calibrated on the basis of analyses performed for the vapor originating from 1 - 5 μl of a solution of 10 μg C(±)P(±)-soman/ml isopropanol pipetted into an empty vial.

Complete inactivation of enzyme activity was checked from gas chromatographic analysis of the four stereoisomers of C(±)P(±)-soman in a 0.2-ml sample of treated blood or tissue homogenate to which 0.6 ml of a stabilizing 0.2 M acetate buffer, pH 3.5, containing 2 mM aluminium sulfate and 0.9 μg neopentyl sarin/ml was added as well as 10 μl of a solution of the internal standard C(±)P(+)-D_{13}-soman (500 ng/ml isopropanol). The mixture was extracted with 3 ml ethyl acetate. Gas chromatographic analysis of the soman stereoisomers in the ethyl acetate phase was performed on a Chirasil-Val fused silica column as described previously (27).

The partition coefficients (P_i) were calculated according to

$$P_i = \frac{C_{ref}(V_{vial}-V_{sal}) - C_i(V_{vial}-V_{sal}-V_i) + (C_{ref}-C_i)P_{sal}V_{sal}}{C_iV_i} \quad (1)$$

in which C_{ref} and C_i are the head-space concentrations in the reference vial and the test vial, respectively; V_{vial}, V_{sal} and V_i are the volumes of the empty vial (9.28 ml), the reference saline (0.75 ml) and the tissue (0.25 ml) in the diluted blood or homogenate, respectively; and P_{sal} is the saline/air partition coefficient. Essentially, the saline/air partition coefficient was also calculated according to this equation which reduces, however, to Equation (2) since the reference vial is empty, i.e., V_{sal} = 0 ml.

$$P_{sal} = \frac{C_{ref}V_{vial} - C_i(V_{vial}-V_i)}{C_iV_i} \quad (2)$$

II.5. *In vitro* determination of covalent binding capacities for C(±)P(-)-soman in blood and tissue homogenates

The method used is based on the procedure applied previously (3) to the separate determination of intact soman, hydrolyzed soman (1,2,2-trimethylpropyl methylphosphonic acid), and covalently bound soman,

which was developed by Harris et al. (29) and by Fleisher and Harris (30). Samples (0.6 g) of blood diluted with a twofold or eightfold weight of 0.01 M veronal buffer, pH 7.5, containing sodium chloride (9 g/l) and saponin (1 mg/ml), 0.9-g samples of homogenates of brain (25%, w/w) and skeletal muscle (10%, w/w), and 0.6-g samples of homogenates of liver (2.5% or 0.2%, w/w), lung (15% or 5%, w/w), and kidney (10% or 2%, w/w), were incubated with C(±)P(-)-^{14}C-soman in a twofold (estimated) molar excess over the total binding capacity, for 15 min at 37 °C. Total radioactivity in the incubate was determined in a 0.1-ml sample. Intact soman still present after the incubation period was extracted from a 0.1-ml sample with 0.225 ml toluene. The radioactivity in the toluene was determined in order to check whether sufficient excess of C(±)P(-)-^{14}C-soman had been used. The residual incubate was acidified with sulfuric acid (6 µl/0.1 g incubate) and subsequently extracted with isobutanol/toluene (1:1, v/v; 0.235 ml/0.1 g incubate) in order to remove hydrolyzed and intact soman. The two phases were mixed with a vortex or, in the case of lung and muscle homogenate, by using a Polytron homogenizer. Radioactivity was then determined in both phases.
Radioactivity was measured by liquid scintillation spectrometry in a Tri-carb 4430 (United Technologies Packard, Downers Grove, IL). Samples of the organic layers were mixed with 12 ml of the liquid scintillation cocktail Hionic-Fluor. Diluted blood and acidified layers obtained after extraction of blood samples were first incubated with a mixture of 1.5 ml of Soluene-350/isopropanol (1:1, v/v) and 0.5 ml of 30% H_2O_2 at room temperature for 10 min and subsequently at 40 °C for 20 min. Next, 12 ml of Hionic-Fluor was added. Homogenates and acidified layers obtained after extraction of homogenates were incubated with 1.5 ml and 3 ml, respectively, of Soluene-350 at room temperature for 30 min and subsequently at 45-50 °C for 2 h. Next, 12 ml of Hionic-Fluor was added. Samples were measured for 20 min or until the standard deviation of the counting measurement was less than 0.5%. Counting efficiencies were determined by external standardization. Calibration curves were made from quenching of ^{14}C-hexadecane by various amounts of isobutanol/-toluene and of diluted blood and liver homogenate (25%, w/w) acidified with sulfuric acid.

II.6. *In vitro* determination of overall rate constants for binding of C(±)P(-)-soman in blood and tissue homogenates

Blood diluted with an eightfold weight of 0.01 M veronal buffer, pH 7.5, containing sodium chloride (9 g/l) and saponin (1 mg/ml); and homogenates of brain (25%, w/w), liver (0.2%, w/w), lung (5%, w/w), kidney (2%, w/w) and skeletal muscle (10%, w/w) were used. A fraction (1-2 g) of diluted blood or homogenate used in a run was incubated (pH 7.5, 37 °C) with C(±)P(-)-^{14}C-soman in an estimated twofold molar excess over the total concentration of binding sites, in order to determine the overall binding capacity. A sample was taken after ca. 15 min. The residual part was incubated (pH 7.5, 37 °C) with C(±)P(-)-^{14}C-soman and C(±)P(+)-soman, both in a concentration which was estimated to be equimolar to that of the binding sites in the diluted blood or homogenate. Samples were taken at various times.

After taking the last sample, additional C(±)P(-)-^{14}C-soman was added to the reaction mixture, thus obtaining a final C(±)P(-)-^{14}C-soman concentration which was 10-fold higher than the estimated overall concentration of the binding sites. After ca. 2 min a sample was taken from this mixture. All samples (ca. 0.4 g or ca. 0.7 g in the case of lung and muscle homogenate) were acidified and subsequently extracted with isobutanol/toluene as described in subsection II.5. Radioactivity was determined in the two phases. Total radioactivity in the incubates was determined in a 0.1-ml sample. Radioactivity measurements were carried out as described in subsection II.5.

II.7. *In vitro* determination of rate constants for hydrolysis of C(-)P(-)-soman in blood and tissue homogenates

The determinations were carried out as described previously for hydrolysis experiments with C(+)P(-)-soman (3). Reactions were started by addition of 1-20 µl of an appropriate C(-)P(-)-soman solution in acetonitrile to 1-4 ml plasma or homogenate equilibrated at pH 7.5 and 37 °C. If necessary, small volumes of 1 N NaOH or 0.1 N HCl were added to maintain the pH at 7.5±0.1. Samples (200 µl) were taken from the reaction mixture after various times to follow the decrease of C(-)P(-)-soman concentration. In order to determine the concentration at zero time of reaction, a sample (2-100 µl) was taken from 0.01 M veronal buffered saline to which the C(-)P(-)-soman solution was added.
For work-up, samples were mixed with 600 µl of the stabilizing acetate buffer containing aluminium sulfate, neopentyl sarin (0.6 µg), and the internal standard C(±)P(+)-D_{13}-soman (4 ng) and passed through a SepPak C_{18} cartridge, as described previously for toxicokinetic measurements (2,27). After washing with 0.8 M aqueous $NaHCO_3$ (3 ml) and water (6 ml), the cartridge was eluted with ethyl acetate (1 ml).
Gas chromatographic analyses of the eluates were performed with a Carlo Erba HRGC 5160 (Mega Series) gas chromatograph equipped with an alkali flame detector and a cold on-column injector. Samples (1-2.5 µl) were injected on a CPSil 8 CB fused silica column (length, 50 m; i.d., 0.32 mm; film thickness, 1.2 µm; Chrompack, The Netherlands). The column was fitted with a retention gap (Carlo Erba) consisting of a piece of uncoated and deactivated fused silica (length, 2 m; i.d., 0.50 mm). For each chromatographic run, the column was heated from 87 to 120 °C at a rate of 20 °C/min. The detector block was held at 250 °C. Carrier gas helium was used at a flow of 1.5 ml/min, and flows of air and hydrogen through the detector were 350 and 35 ml/min, respectively. Make-up gas for the detector was helium, at a flow rate of 40 ml/min. Under these conditions, the retention times of the soman stereoisomers were ca. 8 min.

II.8. Determination of cardiac output and tissue blood flows

The cardiac output and blood flow distribution were determined in anaesthetized, atropinized and artificially ventilated guinea pigs, before and 10 min after i.v. administration of doses of C(±)P(±)-soman corresponding with 2 and 6 LD_{50}. The guinea pigs were weighed

(550-700 g) and were anaesthetized according to the procedure described in subsection II.3. Cannulas were inserted into the left femoral artery and the right carotid artery. The latter cannula was advanced into the left ventricle of the heart. The animal was heparinized via the femoral artery cannula. Next, a tracheal cannula was inserted, the inhalation anaesthesia was terminated, and the animal was rapidly transported to the radionuclide laboratory. The animal was placed under a heating lamp and anaesthetized with a combination of Hypnorm^{R} (1 mg fluanisone and 0.02 mg fentanyl citrate per ml; dosage 1 ml/kg, i.p.) and Nembutal^{R} (60 mg pentobarbital sodium/ml, diluted sixfold with sterile saline before use; dosage 10 mg/kg, i.m). Next, atropine sulfate (1 ml/kg of a solution of 17.4 mg/ml in saline) was administered intraperitoneally. The femoral artery cannula was connected to a peristaltic pump (LKB 2232 MicroPerpex S Pump, Pharmacia LKB Biotechnology, Uppsala, Sweden) via a calibrated polyethylene tube. The pump flow rate was adjusted with water to 0.8 ml/min prior to the experiment. The pump was started about 4.5 min after atropine administration. Thirty seconds later, when a stable flow rate had been attained, a suspension of ^{141}Ce-labelled microspheres (1 ml/kg, corresponding with ca. 600,000 microspheres/kg body weight, 2-3 μCi) in saline, shaken on a whirlmixer and subsequently sonicated up to the moment of injection, was injected via the carotid cannula in a 20-sec time period. Blood was drawn at a constant flow rate for a period of 2 min from the start of the injection of the microspheres. The flow rate was calculated from the length of the calibrated tube filled with blood during the 2-min sampling period. The withdrawn blood volume was replaced with saline. Five minutes later, a dose of C(±)P(±)-soman corresponding with 2 or 6 LD_{50}, 55 μg/kg and 165 μg/kg, respectively, was injected i.v. into the dorsal penis vein (injection volume 1 ml/kg). Ten minutes later, a suspension of ^{103}Ru-labelled microspheres (1 ml/kg, corresponding with ca. 600,000 microspheres/kg body weight, 2-3 μCi) in saline, shaken on a whirlmixer and subsequently sonicated up to the moment of injection, was injected via the same procedure as described above. Ten minutes after injection of the second radionuclide, the animal was killed by intracardial injection of an overdose of Nembutal^{R}. The heart, lungs, diaphragm, liver, kidneys, gastrocnemius et soleus muscle, and brain were collected. Next, the remaining carcass was homogenized with a household meat-grinder, and samples were taken for analysis. The tissue samples were dissolved in two volumes of potassium hydroxide (5 N) during 24 h. A 4-ml sample was taken from these homogenates, which was counted in a gamma-spectrometer (Packard Cobra QC Auto-Gamma Counting System Model 5002/5003, Downers Grove, IL, USA). A data processing program was used for concomitant analysis of ^{141}Ce and ^{103}Ru in the samples.

III RESULTS AND DISCUSSION

III.1. Tissue/blood partition coefficients

Coefficients for partitioning of the C(±)P(±)-soman enantiomeric pairs between tissues and blood were determined *in vitro* according to the method described by Sato and Nakajima (23) and by Gargas et al. (24). Essentially, the partitioning between blood and air, tissue homogenate and air, and between the reference saline, i.e., the medium in which the tissues are homogenated, and air, was determined by gas chromatographic analysis of the concentrations of the enantiomeric pairs of C(±)P(±)-soman in the air phase by using a head-space injection technique. Although the vapour pressure of C(±)P(±)-soman is only approximately 50 Pa at 25 °C (31), its volatility is sufficiently high to apply this method when the sensitive alkali flame NP detector is used. The gas chromatographic resolution of the enantiomeric pairs of C(±)P(±)-soman [C(+)P(+)- + C(-)P(-)-soman and C(+)P(-)- + C(-)P(+)-soman] was achieved by analysis on a capillary CPSil 8 column (2). A representative gas chromatogram of the analyses of the enantiomeric pairs of C(±)P(±)-soman carried out in the air phase equilibrated with blood or homogenates is given in Figure 1.

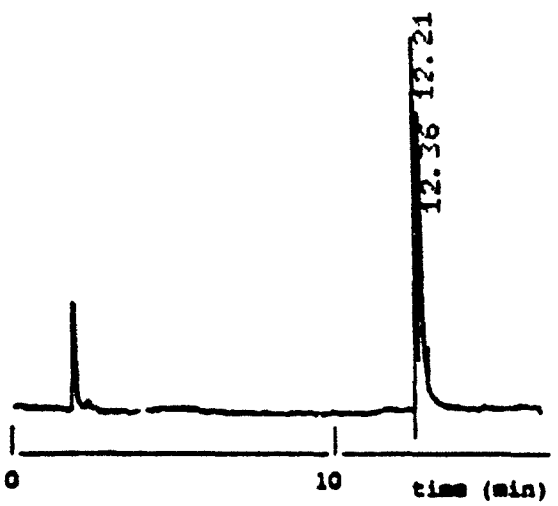

Figure 1. Gas chromatogram of the two enantiomeric pairs of C(±)P(±)-soman determined in a 1-ml sample of the air phase (8.28 ml) equilibrated with guinea pig lung homogenate (25%, 1 ml) which was incubated with 6.65 µl C(±)P(±)-soman after acidification with 2 M acetate buffer, pH 3.3, and pretreatment with crotyl sarin

Initially, it was attempted to inactivate the elimination processes for C(±)P(±)-soman, i.e., covalent binding and hydrolysis, by heat treatment (90 °C, 30 min). The relative concentrations of the

enantiomeric pairs of soman were determined by gas chromatographic analysis of a sample of heat-treated blood and homogenate incubated for two hours with C(±)P(±)-soman at 37 °C in order to check complete inactivation. The ratio for the blood concentrations of [C(-)P(+)- + C(+)P(-)-soman]/[C(+)P(+)- + C(-)P(-)-soman] was increased from 55/45 (the common value in C(±)P(±)-soman, see ref. 17) to 65/35, indicating incomplete inactivation. In a second attempt, blood and the homogenates were acidified by addition of 0.2 M acetate buffer, pH 3.3, (25 µl buffer/ml blood or homogenate). It was previously found that enzymatic hydrolysis of the stereoisomers of soman is inhibited at acidic pH (27), whereas reactions of organophosphates with esterases are generally also slowed down. Gas chromatographic analysis of acidified blood incubated for two hours with C(±)P(±)-soman at 37 °C showed the correct ratio for the enantiomeric pairs indicating complete inactivation. However, hardly any of the four stereoisomers could be found in the acidified homogenates of liver and kidney treated in a similar manner.

In previous experiments we found much higher concentrations of covalent binding sites in liver and kidney than in various other tissues from the guinea pig (3). Therefore, we presumed that the degradation of soman found in acidified homogenates of the two tissues is due to ongoing reaction of soman with covalent binding sites even at the relatively low pH. In the final procedure for inactivation of the elimination processes, we preincubated blood and the homogenates with crotyl sarin. This analogue of soman, which is very unstable in aqueous solution, will be subject to two simultaneous reactions: rapid reaction with covalent binding sites and rapid dealkylation to a nonvolatile methylphosphonofluoridic acid. Additional acidification led to complete inactivation of the elimination processes for C(±)P(±)-soman as determined from gas chromatographic analysis of the four stereoisomers in a sample incubated with the agent for 2 h at 37 °C, provided that a much higher crotyl sarin concentration was used in liver and kidney homogenate than in the other homogenates and in blood.

The partition coefficients obtained are summarized in Table 1. The reproducibility of the determinations was low, although the soman concentrations determined in the head space were far above the minimum detectable concentration and complete inactivation of elimination processes was obtained. The two enantiomeric pairs of soman do not show any stereoselectivity in partitioning between tissue and blood.

In a previous study (3) concentrations of intact, hydrolyzed and covalently bound C(±)P(±)-soman were radiometrically determined in blood and various tissues of the guinea pig 1 h after i.v. administration of 6 LD_{50} C(±)P(±)-^{14}C-soman. The tissue/blood ratios for the concentrations of intact soman calculated from these data are also given in Table 1. These values differ only slightly from the partition coefficients obtained in the present study, except for the much higher *in vivo*

Table 1

Partition coefficients (P)[a] at 37 °C for saline/air and guinea pig blood/air and tissue/air of the enantiomeric pairs [C(+)P(+)- + C(-)P(-)-soman] and [C(+)P(-)- + C(-)P(+)-soman]. Partition coefficients for tissue/blood as calculated from these values are also given. Determinations were carried out by head-space GC analysis in tissue homogenates and diluted blood in which covalent binding sites were occupied with crotyl sarin and enzymatic hydrolysis was precluded by acidification to pH 3.3.

Tissue	C(+)P(+)-/C(-)P(-)-soman		C(+)P(-)-/C(-)P(+)-soman	
	10^{-3}*P tissue/air	P tissue/blood	10^{-3}*P tissue/air	P tissue/blood[b]
Saline	2.1 ± 0.6	-	2.2 ± 0.5	-
Blood	12 ± 6	-	9 ± 4	-
Brain	6 ± 2	0.5	6 ± 3	0.6 (1.1)
Liver	20 ± 9	1.7	20 ± 9	2.1 (2.1)
Kidney	11 ± 3	1.0	11 ± 4	1.2 (7.0)
Fat	6 ± 6	0.5	6 ± 5	0.6
Lung	5 ± 3	0.5	5 ± 3	0.6 (1.2)
Muscle	5 ± 2	0.4	5 ± 2	0.5

[a] Mean ± S.D.; n = 3.

[b] Data in parentheses are ratios of the C(±)P(±)-soman concentrations determined previously in tissue and blood 1 h after i.v. administration of 6 LD_{50} C(±)P(±)-soman (3).

value for the kidney/blood ratio. The relatively high C(±)P(±)-^{14}C-soman concentration found in guinea pig kidney, which was even much more pronounced in rat kidney, is not well understood (6). Gearhart et al. (22) reported tissue/blood partition coefficients for the analogous organophosphate diisopropyl phosphorofluoridate determined in heat treated blood and tissue homogenates. The data for brain/blood, liver/blood and kidney/blood partitioning, i.e., 0.7, 1.5 and 1.6, respectively, are similar to the present values obtained for the soman diastereoisomers.

III.2. Covalent binding capacities for C(±)P(-)-soman in blood and tissue homogenates

The covalent binding capacities for C(±)P(-)-soman in blood and tissue homogenates were determined *in vitro* by using C(±)P(-)-^{14}C-soman. The epimeric pair of stereoisomers was isolated from C(±)P(±)-^{14}C-soman after treatment with rabbit plasma in order to hydrolyze the C(±)P(+)-isomers, in the usual manner (28). A solution of 7.9 μg C(±)P(-)-^{14}C-soman/ml acetonitrile was obtained, which contained 1.1 μg C(±)P(+)-^{14}C-soman/ml. The specific activity of the ^{14}C-labelled isomers was 1.6 GBq/mmol, as determined by mass spectrometry.

Diluted blood and the tissue homogenates were incubated with the C(±)P(-)-^{14}C-soman preparation for 15 min. Our previous experiments

showed that covalent binding is a rapid process, which should be almost complete within a few minutes at the conditions used in the present study. Excess of intact soman and hydrolyzed soman, which is also formed during incubation, were removed by acidification of the incubates and subsequent extraction with isobutanol/toluene (1:1). A similar procedure was previously adopted for the determination of covalently bound C(±)P(±)-^{14}C-soman (3). At the end of the incubation period, a small fraction of the total sample was extracted with toluene removing the intact soman only. The radioactivity in the toluene phase was at least 30% of the total radioactivity added, indicating that a sufficient molar excess of soman was used. The results are summarized in Table 2.

Table 2

Concentration of covalent binding sites[a] (ng soman equivalents/g tissue ± S.D.) as determined from the binding of C(±)P(-)-^{14}C-soman in blood and homogenates of various tissues from guinea pig (pH 7.5, 37 °C). Previous results obtained from *in vitro* experiments with C(+)P(-)-soman and the concentrations of binding sites occupied 1 h after i.v. administration of 6 LD_{50} of C(±)P(±)-^{14}C-soman (3) are included.

Tissue	This study		Previous data on concentrations of covalent binding sites	
	Blood dilution/ homogenate concentration used	Concentration covalent binding sites (ng soman/g)	From *in vitro* experiments (ng soman/g)	After 6 LD_{50} (ng soman/g)
Blood	3-fold	96 ± 5		90 ± 10
Plasma			160 ± 20	
Brain	25%	20 ± 2	20 ± 30	90 ± 10
Lung	15%	115.5 ± 0.4	50 ± 90	370 ± 160
Liver	2.5%	10,400 ± 400	12,000 ± 2000	300 ± 50
Kidney	10%	1,790 ± 50	2,000 ± 600	780 ± 80
Skeletal muscle[b]	10%	22 ± 1	60 ± 60	

[a] N = 3.
[b] Gastrocnemius and soleus muscles.

The present results confirm the relatively high binding capacities of liver and kidneys estimated from previous *in vitro* experiments with C(+)P(-)-soman. The much lower binding capacities found for brain, lung, and skeletal muscle could not be estimated accurately in the previous *in vitro* experiments, which were primarily aimed at determination of the rate of hydrolysis of C(+)P(-)-soman in the homogenates. The concentration of binding sites in blood is about half the concentration in plasma, indicating that most of the binding sites of blood are situated in plasma. Comparison of the present results with the concentrations of binding sites occupied 1 h after

i.v. administration of 6 LD_{50} of C(±)P(±)-^{14}C-soman reveals that the binding sites in blood have been saturated after this intoxication, but that the occupation of the sites in liver and kidney is far from complete even after i.v. administration of 6 LD_{50} of the agent. These observations were described on the basis of "first come, first served" (3, 4). It is remarkable that the concentrations of binding sites occupied in lung and especially in brain after administration of 6 LD_{50} of C(±)P(±)-^{14}C-soman are considerably higher than the total binding capacities found in the homogenates of these tissues. These results need further investigation. It may suggest that not all binding sites in the two tissues are accessible in the homogenates or that a fraction of the binding sites is destroyed during homogenization. However, it should be kept in mind that differences between the results of two series of experiments may occur since outbred guinea pigs were studied.

Additional information on the concentrations of covalent binding sites in diluted blood and tissue homogenates was obtained from experiments in which the rate constants of binding were determined. These results will be discussed in subsection III.3.

III.3. Overall rate constants for binding of C(±)P(-)-soman in blood and tissue homogenates

Essentially, the technique used for determination of the concentrations of overall binding sites (see subsection III.2) was also applied for *in vitro* determination of rate constants for binding by C(±)P(-)-^{14}C-soman. However, equimolar concentrations of binding sites and C(±)P(-)-^{14}C-soman were used, and the degree of binding was estimated at various periods of time after incubation. C(±)P(-)-^{14}C-soman was isolated from C(±)P(±)-^{14}C-soman as described in subsection III.2. A solution of 249 µg C(±)P(-)-^{14}C-soman/ml acetonitrile was obtained, in which no C(±)P(+)-^{14}C-soman could be detected by gas chromatographic analysis. The specific activity was 1.6 GBq/mmol, as determined by mass spectrometry. In previous studies (4) we observed that covalent binding sites are occupied mainly, but not exclusively, by C(±)P(-)-soman after i.v. administration of C(±)P(±)-soman to guinea pigs. Therefore, the determinations of the rate constants for binding by C(±)P(-)-^{14}C-soman were carried out in the presence of an equimolar concentration of C(±)P(+)-soman in order to account for the possible competition of the nontoxic isomers. Furthermore, it was attempted to evaluate the contribution of C(±)P(+)-soman to occupation of the binding sites from the difference between the concentration of all binding sites and the concentration of bound C(±)P(-)-^{14}C-soman determined after addition of C(±)P(-)-^{14}C-soman in a ninefold excess with respect to the totally available binding sites subsequently to taking the last data point.

In all tissue samples, the binding of C(±)P(-)-^{14}C-soman proceeded in two phases, i.e., a rapid occupation of a fraction of the binding sites followed by a very slow reaction or hardly any increase in bound radioactivity. An example of the time course of C(±)P(-)-^{14}C-soman binding in brain homogenate is given in Figure 2. Data points

for the fast binding reactions could only be measured when the reactions in lung, kidney and liver homogenates were carried out in more diluted samples than used for determination of overall concentrations of binding sites. Figure 3 shows an example of binding in a 2% kidney homogenate. Even in ninefold diluted blood, the initial binding was almost complete within 1 min, as shown in Figure 4.

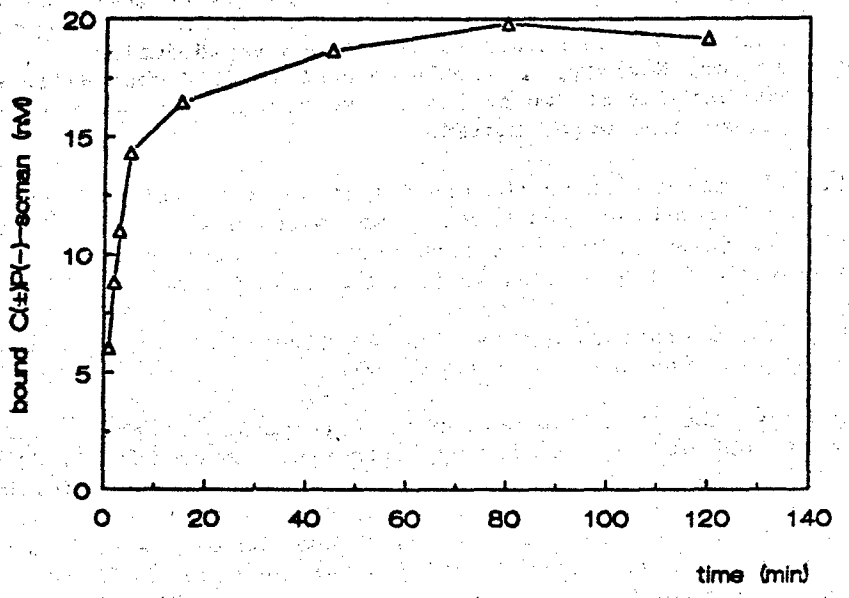

Figure 2. Course of C(±)P(-)-^{14}C-soman binding (pH 7.5, 37 °C) to estimated equimolar sites (27 nM) in brain homogenate (25%) in the presence of an equimolar concentration of C(±)P(+)-soman

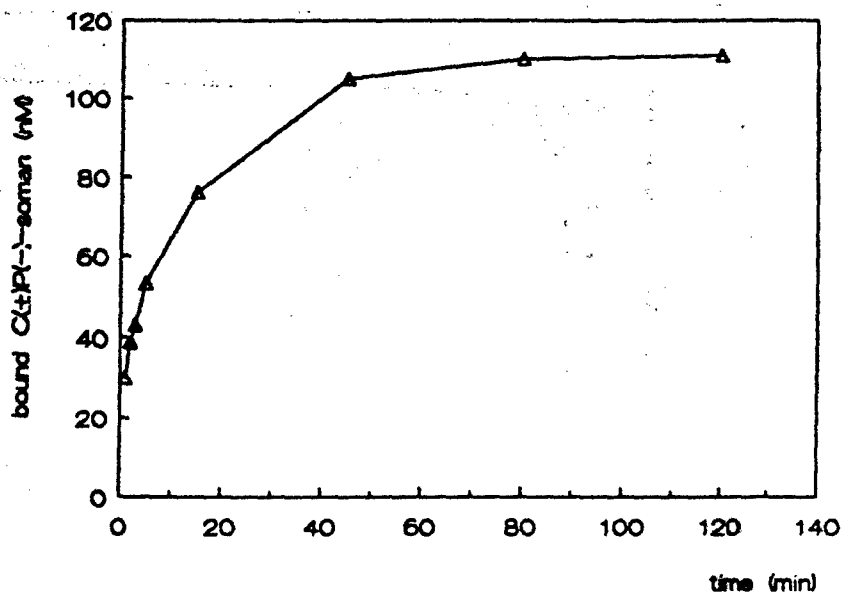

Figure 3. Course of C(±)P(-)-^{14}C-soman binding (pH 7.5, 37 °C) to estimated equimolar sites (200 nM) in kidney homogenate (2%) in the presence of an equimolar concentration of C(±)P(+)-soman

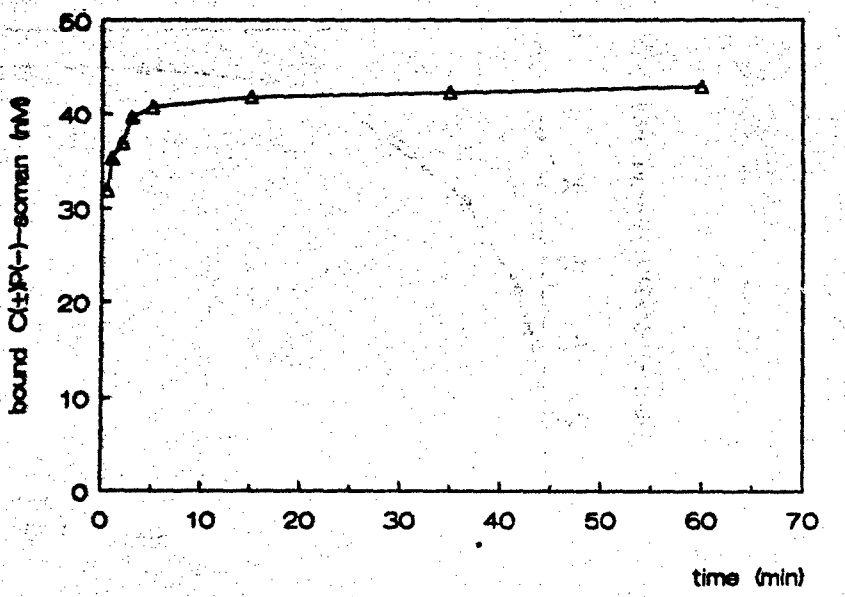

Figure 4. Course of C(±)P(-)-^{14}C-soman binding (pH 7.5, 37 °C) to estimated equimolar sites (60 nM) in ninefold diluted blood in the presence of an equimolar concentration of C(±)P(+)-soman

Rate constants for the fast binding processes were estimated in the following manner. The concentration of rapidly reacting binding sites ([free fast b.s.]$_0$) was estimated from extrapolation of the course of the slow binding in a plot for the concentration of bound C(±)P(-)-^{14}C-soman vs time (see for instance Figures 2-4). Then, the rate constant (k) was calculated from the data points obtained for the fast binding process according to the equation for a bimolecular reaction:

$$\ln\left(\frac{[C(\pm)P(-)\text{-}^{14}C\text{-soman}]_t}{[\text{free fast b.s.}]_t}\right) =$$

$$\ln\left(\frac{[C(\pm)P(-)\text{-}^{14}C\text{-soman}]_0}{[\text{free fast b.s.}]_0}\right) + \{[C(\pm)P(-)\text{-}^{14}C\text{-soman}]_0 - [\text{free fast b.s.}]_0\} * k * t \qquad (3)$$

The calculated rate constants as well as the estimated concentrations of fast reacting binding sites are summarized in Table 3.

Table 3

Rate constants[a] (pH 7.5, 37 °C) for reaction of C(±)P(-)-^{14}C-soman with rapidly reacting binding sites in blood and tissue homogenates from guinea pigs determined in the presence of an equimolar concentration C(±)P(+)-soman. The estimated concentrations[a] of rapidly reacting binding sites in blood and the homogenates are also given.

Tissue[b]	Rate constant for fast binding ($M^{-1}min^{-1}$)	Estimated concentration of rapidly reacting binding sites[c] (ng soman equivalent/g tissue)
Blood (9%)	$\geq 3*10^7$	63±2 (66%)
Brain (25%)	$(2.3±0.5)*10^7$	12±1 (60%)
Lung (5%)	$(9±3)*10^6$	65±6 (56%)
Liver (0.2%)	$(8±2)*10^6$	3700±200 (36%)
Kidney (2%)	$(6±2)*10^5$	1000±100 (53%)
Skeletal muscle[d] (10%)	$(1.4±0.6)*10^7$	6±1 (27%)

[a] Mean ± S.D., n = 4.
[b] The figures within parentheses denote the blood dilution or homogenate concentration at which the rate constants were determined.
[c] The figures within parentheses denote the rapidly reacting binding sites as percentage of the totally available binding sites (see Table 2).
[d] Gastrocnemius and soleus muscles.

More than 50% of the totally available binding sites in blood, brain, lung and kidney show a fast reaction with C(±)P(-)-^{14}C-soman. The percentage of these binding sites in liver and muscle is much less. The relatively high rate constants point to a reaction of C(±)P(-)-^{14}C-soman with enzymes that are highly reactive towards organophosphates, i.e., cholinesterases and carboxylesterases. Since the molar concentrations of cholinesterases are generally much lower than those of carboxylesterases, the latter enzymes are probably mainly involved in the fast binding processes. Rate constants for these enzymes from guinea pig tissues have not been reported in literature so far. The rate constants for fast binding are somewhat lower than the mean values for inhibition (pH 7.7, 25 °C) by C(±)P(-)-^{14}C-soman of bovine erythrocyte AChE and horse serum butyrylcholinesterase, i.e., 7.10^7 and 5.10^7 $M^{-1}min^{-1}$ (32), respectively, but are at least one order of magnitude higher than the rate constant for inhibition (pH 7.7, 25 °C) by C(±)P(±)-soman of horse liver carboxylesterase, i.e., 2.10^5 $M^{-1}min^{-1}$ (33), except for the binding in guinea pig kidney homogenate.

The present results do not allow to evaluate the slow binding phase. In this phase the concentrations of bound C(±)P(-)-^{14}C-soman increase very slightly or do not increase at all. Changes are mostly within the experimental error. Furthermore, hydrolysis of C(±)P(-)-soman

cannot be neglected as a competitive reaction for the slow binding, complicating the kinetic analysis of the ongoing reactions. Half-life times for hydrolysis of ca. 300 min in liver homogenate and of ca. 70-130 min in the other samples were extrapolated from our hydrolysis data (see subsection III.4), taking into account the concentrations of homogenates and of diluted blood at which the binding experiments were performed.

As already mentioned, C(±)P(-)-^{14}C-soman was added to the reaction mixture in a ninefold excess with respect to the totally available binding sites subsequently to taking the sample for the last data point, in order to evaluate the contribution of C(±)P(+)-soman to the binding. Surprisingly, the concentrations of bound C(±)P(-)-^{14}C-soman in the samples taken after addition of C(±)P(-)-^{14}C-soman at the end of the binding experiments for blood, brain, kidney and muscle were higher than the concentrations of total binding sites determined simultaneously in the same sample of diluted blood or homogenate as a reference value by using a twofold excess of C(±)P(-)-^{14}C-soman without adding C(±)P(+)-soman. Results are summarized in Table 4 (third and second column, respectively) together with the other values for the concentration of binding sites obtained in the present study. Consequently, the present investigations do not allow to evaluate the contribution of C(±)P(+)-soman to the occupation of binding sites. However, the results of the investigations for liver and lung homogenate indicate that at the end of the binding experiment a fraction of the binding sites was no longer available for reaction with C(±)P(-)-^{14}C-soman and was, consequently, occupied by C(±)P(+)-soman (Table 4).

The survey of the values for the concentrations of binding sites obtained under various conditions (Table 4) suggests that these concentrations may depend on the C(±)P(-)-^{14}C-soman concentration used in the experiment, as is obvious from comparison of the values presented in the first and third columns as well as in the second and fourth columns. The data presented in the first column were obtained from the investigations on the rates of binding as the data points after 15 min of reaction. These experiments were performed in the presence of an equimolar concentration of C(±)P(+)-soman. Subsequent addition after 1-3 h of C(±)P(-)-^{14}C-soman up to a 10-fold higher concentration increases the concentration of bound radioactivity considerably (column 3). The values given in the fourth column are derived from subsection III.2. These values were obtained from experiments carried out with less diluted blood and with more concentrated homogenates and, consequently, with higher C(±)P(-)-^{14}C-soman concentrations than used in the experiments described in this subsection, except for brain and muscle homogenate. As the expected concentrations of binding sites in brain and muscle homogenate were higher than actually measured, higher C(±)P(-)-^{14}C-soman concentrations were also used in the experiments described in subsection III.2 for these homogenates than in the determinations of a reference value for the total binding sites carried out simultaneously with the experiments on the rate of binding (Table 4, second column). Comparison of the values presented in the second and

fourth columns reveals a larger amount of bound soman in liver, kidney and skeletal muscle homogenate when using a higher concentration of C(±)P(-)-^{14}C-soman.

Table 4

Amounts of C(±)P(-)-^{14}C-soman covalently bound in blood and homogenates of various tissues from guinea pig (pH 7.5, 37 °C) after incubation with various C(±)P(-)-^{14}C-soman concentrations, indicated as values relative to the concentration of binding sites,[a] at various experimental conditions (A-D)[b] for 15 min (A,B,D) or 2 min (C)

Tissue[c]	Bound C(±)P(-)-^{14}C-soman (ng/g tissue ± S.D.)[d] measured with various C(±)P(-)-^{14}C-soman concentrations at conditions A-D			
	1[a] A[b]	2[a] B[b]	10[a] C[b]	variable[a,e] D[b]
Blood (9x/3x)	66±2	89±7	93±11	96±5 (6)
Brain (25%)	12±1	20±2	22±3	20±2 (3)
Lung (5%/15%)	64±8	113±20	100±13	115.5±0.4 (4)
Liver (0.2%/2.5%)	3600±700	8300±700	6000±400	10400±400 (29)
Kidney (2%/10%)	750±70	1300±200	1500±200	1790±50 (11)
Skeletal muscle[f] (10%)	5.0±0.6	7±1	17±3	22±1 (7)

[a] Absolute C(±)P(-)-^{14}C-soman concentrations used at condition A, i.e., at relative concentrations of 1, were 10.7, 5, 6, 21, 36, and 2.2 ng/ml for reaction with diluted blood and with the homogenates of brain, lung, liver, kidney and muscle, respectively.

[b] A-C: highest blood dilution or lowest homogenate concentration; D: lowest blood dilution or highest homogenate concentration. A: C(±)P(+)-soman added at relative concentration of 1; C: as A but with a subsequent addition of C(±)P(-)-^{14}C-soman up to a relative concentration of 10 after 1 h, 2 h (kidney) or 3 h (muscle); B and D: no C(±)P(+)-soman added.

[c] The figures in parentheses denote the blood dilutions and homogenate concentrations used.

[d] N = 4 for A-C; n = 3 for D.

[e] Data from Table 2; the figures in parentheses denote the relative C(±)P(-)-^{14}C-soman concentrations.

[f] Gastrocnemius and soleus muscles.

The concentrations of C(±)P(-)-^{14}C-soman bound in lung and muscle homogenate at the conditions of the experiments summarized in third column are lower than the values obtained from the experiments described in subsection III.2 (fourth column), although the C(±)P(-)-^{14}C-soman concentrations used in the former experiments were higher. This is probably due to concurrent binding by C(±)P(+)-soman which was initially present in an equimolar concentration (vide supra). The presence of C(±)P(+)-soman has probably also decreased the values for liver and kidney homogenates given in the third column. Summarizing,

C(±)P(-)-^{14}C-soman concentration should be sufficiently high to occupy also the less reactive binding sites within the incubation period (15 min). The survey of Table 4 indicates that the best estimates for the total concentrations of binding sites are the values presented in subsection III.2 (Table 2 and Table 4, fourth column).

The dependence of the extent of binding on the concentration of C(±)P(-)-^{14}C-soman was studied in a separate experiment with the homogenates in which clearly different results were found at the various conditions used in the experiments summarized in the second and fourth columns of Table 4, i.e., liver, kidney and muscle. The results of this experiment, given in Table 5, clearly show that more C(±)P(-)-^{14}C-soman is bound when the homogenates are treated with a higher concentration of the isomers. For comparison of the results presented in the Tables 4 and 5, the same absolute C(±)P(-)-^{14}C-soman concentrations were set equal to 1. The somewhat higher concentrations of bound soman found in kidney and muscle homogenate than found in previous experiments at corresponding C(±)P(-)-^{14}C-soman concentrations (compare Tables 5 and 4) are probably due to the age of the homogenates, which had been stored at -20 °C for several months. The increased levels of binding sites upon storage which we also observed in other studies (unpublished results) might be caused by bacterial contamination.

Table 5

Amounts of C(±)P(-)-^{14}C-soman covalently bound in homogenates of guinea pig liver, kidney and skeletal muscle (pH 7.5, 37 °C) after incubation with various C(±)P(-)-^{14}C-soman concentrations, indicated as values relative to the concentration of binding sites,[a] for 15 min

Tissue[b]	Bound C(±)P(-)-^{14}C-soman (ng/g tissue)[c]		
	Concentrations of C(±)P(-)-^{14}C-soman relative to that of the binding sites		
	2	4	10
Liver (0.2%)	6900-8300	8100-8700	8800-10000
Kidney (2%)	1800-1900	2300-2400	3000-3100
Skeletal muscle[d] (10%)	10-11	15-15	34-35

[a] The absolute C(±)P(-)-^{14}C-soman concentrations set equal to 1 in Table 4 were also taken as a reference value for these data, i.e., 21, 36 and 2.2 ng/ml for reaction in liver, kidney and muscle homogenate, respectively.
[b] The concentration of homogenate is given within parentheses.
[c] Results of duplicate experiments.
[d] Gastrocnemius and soleus muscle.

According to our description of the binding processes, the differences between the values given in the second column of Table 4

and the estimated values for the concentrations of rapidly reacting binding sites (Table 3) should represent the concentrations of slowly reacting binding sites occupied by C(±)P(-)-^{14}C-soman when incubated with a twofold molar excess of the agent for 15 min. From these differences, rate constants for slow binding were calculated according to the equation for a bimolecular reaction, analogous to Equation 3. The rate constants, which ranged from $1*10^5$ to $6*10^6$ $M^{-1}min^{-1}$, were subsequently used to calculate the degree of slow binding that should be achieved after incubation with C(±)P(-)-^{14}C-soman at an equimolar concentration relative to the totally available binding sites (conditions for the first column of Table 4) for 1 h, 2 h (kidney) or 3 h (muscle). These calculations predict a much higher occupation of the slowly reacting binding sites by C(±)P(-)-^{14}C-soman (> 50%, or 40% for the muscle homogenate) than we actually found in our kinetic experiments, in which less than 25% of the slow binding sites had reacted (see Figures 2-4). These results point to a more complex mechanism of C(±)P(-)-soman binding than initially assumed. A detailed study will be needed in which the effect of the C(±)P(-)-soman concentration, the blood dilution and the homogenate concentration on the degree of binding and on the relative concentrations of rapidly and slowly reacting binding sites is investigated.

The results presented in this subsection indicate that incomplete occupation of binding sites even after treatment with a twofold excess of C(±)P(-)-^{14}C-soman is due to a very low reactivity of a fraction of the binding sites. However, this limited study does not provide information, which allows to speculate whether the low reactivity is due to difficult accessibility of the binding sites that is improved by increasing the homogenate and/or C(±)P(-)-soman concentration, for instance by affecting the degree of dissociation of aggregates of the molecules containing the binding sites.

III.4. Rate constants for hydrolysis of C(-)P(-)-soman in blood and tissue homogenates

The decrease of the C(-)P(-)-soman concentration in plasma and tissue homogenates proceeded in two phases. In our previous study on the hydrolysis of C(+)P(-)-soman (3), we made similar observations and attributed the rapid initial removal of a fraction of the soman isomer to covalent binding. In the present experiments this process was complete within a few minutes. Our toxicokinetic studies indicated that hydrolysis of C(±)P(-)-soman will mainly contribute to its elimination subsequent to the initial rapid concentration decay following i.v. administration of 2-6 LD_{50} C(±)P(±)-soman. After this initial decay, the concentration of C(±)P(-)-soman in blood was ca. 40 ng/ml. Therefore, the initial C(-)P(-)-soman concentration was chosen in such a way that the concentration decrease of C(-)P(-)-soman from 40 to 20 ng/ml was within the second phase.

Upon incubation of C(-)P(-)-soman in plasma and tissue homogenates, an almost immediate concentration decrease was observed followed by a further decrease according to first-order kinetics (see Figure 5 for

an example), except for the hydrolysis in kidney homogenate. Apparently, covalent binding proceeds somewhat slower in the latter homogenate, which is in accordance with the results presented in subsection III.3 (see Table 3). The results obey first-order kinetics after an initial reaction time of ca. 9 min (Figure 6). The half-life times evaluated for the first-order reactions are collected in Table 6.

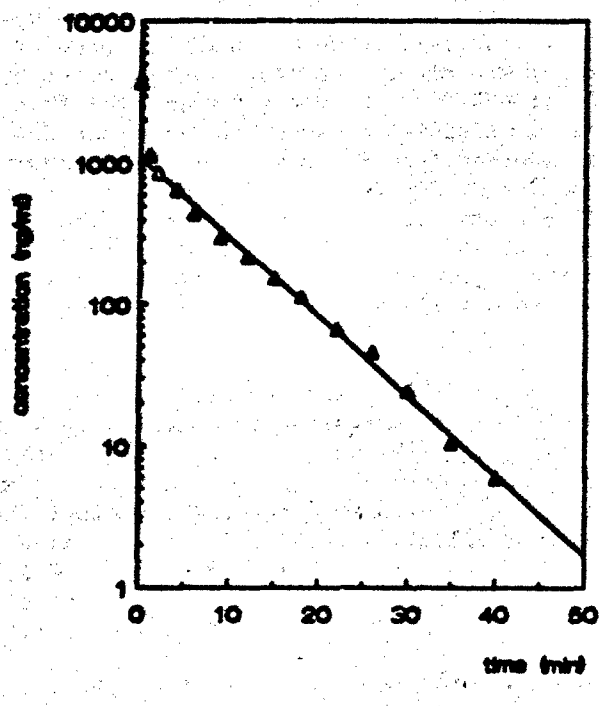

Figure 5. Semilogarithmic plot of the decrease of C(-)P(-)-soman concentration in a 25% homogenate of guinea pig liver at pH 7.5 and 37 °C. The line represents the optimal fit according to linear regression to all data except the data point at time point zero.

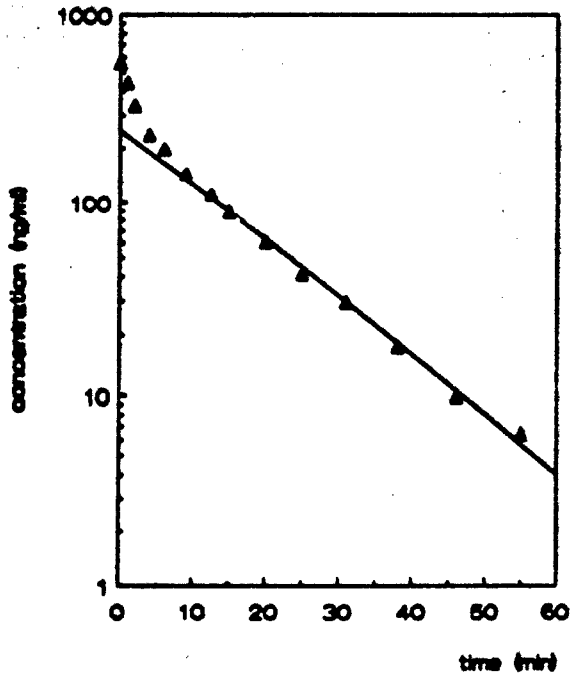

Figure 6. Semilogarithmic plot of the decrease of C(-)P(-)-soman concentration in a 25% homogenate of guinea pig kidney at pH 7.5 and 37 °C. The line represents the optimal fit according to linear regression to the data points from time point 9 min.

Table 6

Half-life times[a] for *in vitro* hydrolysis of C(-)P(-)-soman in plasma and 25% homogenates[b,c] of various tissues from guinea pig, at pH 7.5 and 37 °C. Previous *in vitro* results (3) on the time period[a] needed for decrease of the C(+)P(-)-soman concentration from 40 to 20 ng/ml [t(40-20)] are included.

Tissue	Half-life time C(-)P(-)-soman (min)	t(40-20) C(+)P(-)-soman (min)
Plasma	7.0 ± 0.2 (4)	7 ± 1
Brain	70 ± 6 (5)	72 ± 14
Lung	41 ± 5[b]	29 ± 7
Liver	5.2 ± 0.3	2.4 ± 0.5
Kidney	10 ± 1 (4)	11 ± 1
Skeletal muscle[c,d]	77 ± 7	81 ± 15 (4)

[a] Means ± S.D., n = 3 unless otherwise noted in parentheses.
[b] The lung homogenate used in the present study was 15%.
[c] The homogenate of the skeletal muscle was 10%.
[d] Gastrocnemius and soleus muscle.

The results obtained for the two P(-)-isomers are rather similar. The slower hydrolysis of C(-)P(-)-soman in lung homogenate is probably due to the use of a more diluted homogenate (15% instead of 25%) in the present experiments. The catalytic activities in the homogenates of tissues that are considered to be target organs for the toxic action of soman, i.e., brain and skeletal muscle, are much lower than in plasma and the other homogenates, which are part of the central compartment.

III.5. Cardiac output and tissue blood flows

Tissue blood flows were determined from the distribution of radioactively labelled microspheres as described by Peeters et al. (25). The time needed to install the cannulas varied considerably between the animals. Therefore, we decided to perform the surgical manipulations under inhalation anaesthesia, which is very suitable for controlled, prolonged anaesthesia. Since there are no facilities for inhalation anaesthesia in the radionuclide lab, injection of anaesthetics was necessary subsequent to halothane anaesthesia. Ketamine was our first choice as an injectable anaesthetic. Unfortunately, several animals died shortly after i.m. administration of this compound. It seems that halothane anaesthesia followed by ketamine is not very well tolerated by guinea pigs. We replaced ketamine with the combination of Hypnorm^{R} (1 ml/kg, i.m.) and Nembutal^{R} (10 mg/kg, i.p.), which in previous toxicokinetic experiments appeared to be very suitable for guinea pigs, in particular subsequent to halothane anaesthesia (5).

We encountered considerable difficulties in advancing the femoral artery cannula into the abdominal aorta, as was described by Peeters et al. (25), leading to a high failure incidence due to piercing of the artery with the tip of the cannula. After extensive practice our dexterity improved, but nevertheless, only 1 out of 3 cannulations was successful. In discussions with scientists experienced in this technique with small animals, it was suggested that positioning of the tip of the cannula in the aorta is not essential to obtain a reference flow rate. As long as the sample is drawn from a peripheral artery at a constant flow rate, which must not be unrealistically high for this artery, an adequate reference flow rate is obtained.

We decided not to use a separate control group, as originally proposed, but made two subsequent injections of differently labelled (^{141}Ce and ^{103}Ru) microspheres to each animal, which has the advantage that each animal can serve as its own control. Since only 2-3% of the capillaries are blocked upon each injection, it is possible to perform more than one injection per animal, without too much impairment of the blood flows. Unfortunately, in some cases the blood flow from the femoral artery cannula was not constant or too low during either the first or the second sampling, necessitating additional experiments.

The tissue blood flow rate was calculated from

$$\text{tissue flow rate} = \frac{\text{dpm in the tissue}}{\text{dpm in reference sample}} * \text{reference flow rate} \quad (4)$$

as reported by Peeters et al. (25). The cardiac output was calculated as the sum of all tissue flows.

The values obtained for the tissue blood flow rates and cardiac output in the individual animals are presented in Tables 7 and 8.

The mean values for the cardiac output before and after administration of C(±)P(±)-soman are presented in Table 9.

The mean control value for cardiac output comprises all successful experiments (n = 12) with the ^{141}Ce-labelled microspheres. Our mean control value is about twofold lower than that reported by Peeters et al. (25). However, the latter study was performed with animals which were neither anaesthetized nor atropinized. Furthermore, these authors used female guinea pigs weighing ca. 1 kg, whereas our male animals weighed 550-700 g. In Table 9, control data are also presented for both soman dose groups separately. These data were obtained from the studies in which the injections of both labelled microspheres were successful.

The data in Table 9 indicate that the administration of 2 and 6 LD_{50} C(±)P(±)-soman does not alter the cardiac output at 10 min after administration, which is in agreement with the findings of Maxwell et al. (10) for 0.84 LD_{50} C(±)P(±)-soman in the rat.

Table 7

Blood flow rates to various tissues and cardiac output in anaesthetized, atropinized and artificially ventilated guinea pigs before and after i.v. administration of 2 LD_{50} of C(±)P(±)-soman

Tissue	Blood flow rate ($ml.min^{-1}.kg^{-1}$)													
	Guinea pig 2-1 (690)[a]		Guinea pig 2-2 (555)		Guinea pig 2-3 (583)		Guinea pig 2-4 (587)		Guinea pig 2-5 (571)[a]		Guinea pig 2-6 (577)		Guinea pig 2-7 (690)	
	Before Soman	After Soman	Before Soman	After Soman[b]	Before Soman	After Soman	Before Soman	After Soman	Before Soman[b]	After Soman	Before Soman	After Soman	Before Soman	After Soman
Brain	2.12	1.93	1.68	-	1.97	2.69	0.81	0.81	-	1.01	3.98	3.35	1.69	3.41
Lung	18.62	5.54	16.15	-	3.21	4.32	1.23	0.14	-	1.13	10.38	5.29	6.09	5.94
Muscle	4.28	0.41	0.63	-	0.31	0.43	0.08	0.25	-	0.30	0.06	0.06	0.10	0.12
Diaphragm	0.04	0.02	0.19	-	0.19	0.26	0.09	0.14	-	0.12	0.17	0.16	0.16	0.30
Carcass	99.8	69.3	63.2	-	63.4	72.4	27.09	32.99	-	40.92	113.6	91.3	82.4	101.5
Kidney	8.5	5.57	13.45	-	7.00	5.31	1.08	0.28	-	0.65	9.59	4.08	10.40	8.66
Heart	3.31	3.95	49.6	-	1.59	3.09	4.34	3.71	-	0.65	6.76	4.77	5.07	3.78
Liver	1.03	0.88	1.46	-	0.85	1.33	0.65	0.33	-	0.48	1.40	1.77	1.18	1.21
Cardiac output	137.7	87.7	146.4	-	78.6	89.8	35.4	38.7	-	44.7	145.9	110.8	107.4	124.9

[a] Weight (g).
[b] Flow rates could not be calculated due to failed reference sampling.

Table 8

Blood flow rates to various tissues and cardiac output in anaesthetized, atropinized and artificially ventilated guinea pigs before and after i.v. administration of 6 LD_{50} of C(±)P(±)-soman

Tissue	Blood flow rate (ml.min^{-1}.kg^{-1})													
	Guinea pig 6-1 (595)[a]		Guinea pig 6-2 (650)		Guinea pig 6-3 (636)		Guinea pig 6-4 (652)		Guinea pig 6-5 (561)[a]		Guinea pig 6-6 (664)		Guinea pig 6-7 (555)	
	Before Soman[b]	After Soman	Before Soman	After Soman[b]	Before Soman	After Soman	Before Soman	After Soman	Before Soman	After Soman	Before Soman	After Soman	Before Soman	After Soman
Brain	-	3.02	1.09	-	0.80	2.11	2.71	1.80	1.66	3.29	2.15	2.39	0.01	0.03
Lung	-	2.07	1.15	-	3.62	4.48	30.32	7.05	2.94	5.88	175.4	6.32	16.19	5.98
Muscle	-	0.23	0.10	-	0.40	0.37	0.64	0.32	0.05	0.14	0.16	0.23	6.37	5.38
Diaphragm	-	0.33	0.11	-	0.11	0.22	0.45	0.15	0.18	0.29	0.12	0.10	0.18	0.42
Carcass	-	88.3	32.4	-	59.5	61.5	132.3	80.2	91.9	115.0	73.2	76.5	207.3	405.2
Kidney	-	8.46	2.22	-	7.31	3.50	7.82	3.91	5.12	4.05	9.74	3.53	42.5	78.8
Heart	-	4.78	2.22	-	0.77	0.61	4.26	1.85	2.68	6.37	7.47	3.71	0.01	0.01
Liver	-	1.03	0.55	-	0.93	1.34	1.66	1.42	0.49	0.63	1.40	1.21	22.01	18.17
Cardiac output	-	108.2	39.9	-	73.4	74.1	180.1	96.7	105.0	135.6	269.6	93.9	294.6	513.9

[a] Weight (g).
[b] Flow rates could not be calculated due to failed reference sampling.

Table 9
Mean cardiac output (± s.e.m.) of anaesthetized, atropinized and artificially ventilated guinea pigs, weighing 550-700 g, before and 10 min after administration of 2 and 6 LD_{50} C(±)P(±)-soman

	Cardiac output ($ml.min^{-1}.kg^{-1}$)
Control (n=12)	134 ± 24
Control for the 2 LD_{50} group (n=5)	101 ± 20
After 2 LD_{50} C(±)P(±)-soman (n=5)	90 ± 15
Control for the 6 LD_{50} group (n=5)	185 ± 44
After 6 LD_{50} C(±)P(±)-soman (n=5)	182 ± 84
Literature[a] (n=5)	280 ± 38

[a] Data taken from Peeters et al. (25)

The mean values calculated for the distribution of the cardiac output are presented in Table 10.

Table 10
Distribution (percentage ± s.e.m.) of the cardiac output in anaesthetized, atropinized and artificially ventilated guinea pigs, weighing 550-700 g, before and 10 min after administration of 2 and 6 LD_{50} C(±)P(±)-soman

Tissue	Control (n = 12)	2 LD_{50} (n = 6)	6 LD_{50} (n = 6)	Literature[a]
Kidney	7.1 ± 1.0	4.0 ± 1.0	6.4 ± 1.9	14.3 ± 0.5
Liver	1.6 ± 0.5	1.2 ± 0.2	1.6 ± 0.4	0.3 ± 0.1
Lung	7.1 ± 1.4	3.9 ± 0.9	4.6 ± 1.0	5 ± 1
Brain	1.8 ± 0.2	2.6 ± 0.2	2.1 ± 0.4	2.1 ± 0.5
Muscle	0.6 ± 0.3	0.4 ± 0.1	0.4 ± 0.2	n.r.[b]
Diaphragm	0.16 ± 0.03	0.23 ± 0.06	0.20 ± 0.04	n.r.
Heart	6.2 ± 2.7	4.4 ± 1.1	2.6 ± 0.8	4.0 ± 0.5
Carcass	77.8 ± 1.5	83.0 ± 2.0	82.1 ± 0.8	74.3[c]

[a] Data taken from Peeters et al. (25).
[b] Not reported.
[c] The literature data are not reported on exactly the same carcass as studied in our experiments.

The control data are in reasonable agreement with those reported in literature. The blood flow to the kidneys is about twofold lower in our animals, which may be due to the anaesthesia. No dramatic changes occur in the blood flow distribution at 10 min after administration

of C(±)P(±)-soman. Some trends appear to be present in kidney, lung, brain, and heart, but there are no distinct changes due to the high standard errors of the data. This seems to disagree with the findings of Maxwell et al. (10) after 0.84 LD_{50} in the rat, in which study the blood flows to the brain, lung, muscle, kidney and heart changed significantly. However, the rats were <u>not</u> atropinized in their study. Apparently, atropine reduces the influence of C(±)P(±)-soman on the tissue blood flow distribution. It cannot be ruled out, however, that considerable changes in cardiac output and/or blood flow distribution take place during the initial 10-min period of C(±)P(±)-soman intoxication.

IV. CONCLUSIONS

1. The present study provides toxicant-specific parameters of C(±)P(-)-soman as well as tissue blood flow and cardiac output needed for physiologically based modelling of C(±)P(±)-soman intoxication of guinea pigs.

2. C(±)P(±)-soman is sufficiently volatile to determine the partition coefficients of its stereoisomers for blood/air and tissue homogenate/air by gas chromatographic analysis in the air phase, using a head-space injection technique.

3. The heat treatment procedure reported in literature for inactivation of elimination processes for organophosphate in blood samples and tissue homogenates was not suitable for C(±)P(±)-soman.

4. Elimination of C(±)P(±)-soman by covalent binding in blood samples and tissue homogenates can effectively be stopped by pretreatment with crotyl sarin, which rapidly reacts with covalent binding sites, but is also rapidly dealkylated to nonvolatile methylphosphonofluoridic acid.

5. Only a fraction of the available covalent binding sites in guinea pig blood and tissue homogenates reacts rapidly with C(±)P(-)-soman.

6. Due to a very low reactivity of the remaining fraction of the binding sites the determination of the total concentration should be carried out with a sufficiently high concentration of C(±)P(-)-soman.

7. Indications were obtained for a contribution of C(±)P(+)-soman to covalent binding in guinea pig liver and kidney homogenate when treated with C(±)P(±)-soman; the method used was not adequate to evaluate the contribution of C(±)P(+)-soman to covalent binding in homogenates of other tissues or in blood.

8. The rates of hydrolysis of C(+)P(-)-soman and C(-)P(-)-soman in guinea pig plasma and in various tissue homogenates are similar.

9. Both hydrolytic activity and binding capacity for C(±)P(-)-soman in target tissues (brain, muscle) of the guinea pig are lower than in most of the tissues participating in central elimination.

10. Intravenous administration of 2 and 6 LD_{50} C(±)P(±)-soman to anaesthetized, atropinized and artificially ventilated guinea pigs does not affect the cardiac output determined 10 min after intoxication.

11. No significant changes in blood flow distribution over various tissues was observed 10 min after intravenous administration of C(±)P(±)-soman to anaesthetized, atropinized and artificially ventilated guinea pigs.

12. The influence of covalent binding of C(±)P(-)-soman on its elimination *in vivo* is not fully understood; in this connection, it may be worthwhile to study *in vitro* whether the concentrations of C(±)P(-)-soman and/or of tissue homogenates affect the concentration of totally available binding sites and the ratio between rapidly and slowly reacting binding sites.

13. Further investigations are needed to establish whether changes in cardiac output and/or blood flow distribution take place during the initial 10-min period of intoxication with C(±)P(±)-soman.

REFERENCES

1. BENSCHOP, H.P., BIJLEVELD, E.C., DE JONG, L.P.A., VAN DER WIEL, H.J. AND VAN HELDEN, H.P.M. (1987) Toxicokinetics of the four stereoisomers of the nerve agent soman in atropinized rats - Influence of a soman simulator. Toxicol. Appl. Pharmacol. 90, 490-500.
2. BENSCHOP, H.P. AND DE JONG, L.P.A. (1987) Toxicokinetics of the four stereoisomers of soman in the rat, guinea pig, and marmoset. Annual/final report grant DAMD17-85-G-5004, NTIS AD-A199 573.
3. BENSCHOP, H.P. AND DE JONG, L.P.A. (1990) Toxicokinetic investigations of C(±)P(±)-soman in the rat, guinea pig and marmoset at low dosages - Quantification of elimination pathways. Final report grant DAMD17-87-G-7015, NTIS AD-A210 426.
4. BENSCHOP, H.P. AND DE JONG, L.P.A. (1991) Toxicokinetics of soman: Species variation and stereospecificity in elimination pathways. Neurosci. Biobehav. Rev. 15, 73-77.
5. DE JONG, L.P.A., LANGENBERG, J.P., VAN DIJK, C. DUE, A. AND BENSCHOP, H.P. (1991) Studies on the toxicokinetics of the soman stereoisomers in guinea pigs: stereoselective elimination and toxicokinetics after subcutaneous administration. Proceedings of the Meeting of AC/243 Panel 8 RSG 3, Grenoble, 8-12 April.
6. DE JONG, L.P.A., BENSCHOP, H.P., DUE, A., VAN DIJK, C., TRAP, H.C., VAN DER WIEL, H.J. AND VAN HELDEN, H.P.M. (1992) Soman levels in kidney and urine following administration to rat, guinea pig, and marmoset. Life Sci. 50, 1057-1062.
7. BENSCHOP, H.P. AND VAN HELDEN, H.P.M. (1991) Toxicokinetics of inhaled soman and sarin in guinea pigs. Midterm report grant DAMD17-90-Z-0034.
8. LANGENBERG, J.P., TRAP, H.C., SPRUIT, W.E.T , DUE, A.H., BENSCHOP, H.P., VAN DER WIEL, H.J., BERGERS, W.W.A. AND VAN HELDEN, H.P.M. (1992) Inhalation toxicokinetics of C(±)P(±)-soman in the guinea pig. Proceedings of the Meeting of AC/243 Panel 8 RSG 3, Medicine Hat, 21-24 September.
9. REYNOLDS, M.L., LITTLE, P.J., THOMAS, B.F., BAGLEY, R.B. AND MARTIN, B.R. (1985) Relationship between the biodisposition of [^{3}H]soman and its pharmacological effects in mice. Toxicol. Appl. Pharmacol. 80, 409-420.
10. MAXWELL, D.M., LENZ, D.E., GROFF, W.A., KAMINSKIS, A. AND FROELICH, H.L. (1987) The effects of blood flow and detoxification on in vivo cholinesterase inhibition by soman in rats. Toxicol. Appl. Pharmacol. 88, 66-76.
11. MAXWELL, D.M., VLAHACOS, C.P. AND LENZ, D.E. (1988) A pharmacodynamic model for soman in the rat. Toxicol. Lett. 43, 175-188.
12. MARTIN, B.R. (1985) Biodisposition of [^{3}H]diisopropylfluorophosphate in mice. Toxicol. Appl. Pharmacol. 77, 275-284.
13. LITTLE, P.J., REYNOLDS, M.L., BOWMAN, E.R. AND MARTIN, B.R. (1986) Tissue disposition of [^{3}H]sarin and its metabolites in mice. Toxicol. Appl. Pharmacol. 83, 412-419.
14. SCIMECA, J.A., LITTLE, P.J. AND MARTIN, B.R. (1985) Relationship between the pharmacological effects and the biodisposition of

[^{3}H]diisopropylfluorophosphate in mice after inhalation. Toxicol. Appl. Pharmacol. 79, 502-510.
15. SCIMECA, J.A. AND MARTIN, B.R. (1988) The disposition of [^{3}H]diisopropylfluorophosphate in guinea pigs after inhalation. Drug Metab. Dispos. 16, 534-539.
16. LANGENBERG, J.P., DE JONG, L.P.A. AND BENSCHOP, H.P. (1990) The influence of the rate of spontaneous reactivation on the efficacy of pretreatment against soman poisoning. Proceedings of the Meeting of AC/243 Panel 8 RSG 3, The Hague, 13-17 November 1989, pp. 8.1-8.6.
17. BENSCHOP, H.P. AND DE JONG, L.P.A. (1988) Nerve agent stereoisomers: analysis, isolation, and toxicology. Acc. Chem. Res. 21, 368-374.
18. DEDRICK, R.L., FORRESTER, D.D., CANNON, J.N., EL DAREER, S.M. AND MELLETT, L.B. (1973) Pharmacokinetics of 1-β-D-arabinofuranosylcytosine (ARA-C) deamination in several species. Biochem. Pharmacol. 22, 2405-2417.
19. KING, F.G., DEDRICK, R.L., COLLINS, J.M., MATTHEWS, H.B. AND BIRNBAUM, L.S. (1983) Physiological model of the pharmacokinetics of 2,3,7,8-tetrachlorodibenzofuran in several species. Toxicol. Appl. Pharmacol. 67, 390-400.
20. RAMSEY, J.C. AND ANDERSEN, M.E. (1984) A physiologically based description of the inhalation pharmacokinetics of styrene in rats and humans. Toxicol. Appl. Pharmacol. 73, 159-175.
21. ANDERSEN, M.E., CLEWELL III, H.J., GARGAS, M.L., SMITH, F.A. AND REITZ, R.H. (1987) Physiologically based pharmacokinetics and the risk assessment process for methylene chloride. Toxicol. Appl. Pharmacol. 87, 185-205.
22. GEARHART, J.M., JEPSON, G.W., CLEWELL III, H.J., ANDERSEN, M.E. AND CONOLLY, R.B. (1990) Physiologically based pharmacokinetics and the pharmacodynamic model for inhibition of acetylcholinesterase by diisopropyl fluorophosphate. Toxicol. Appl. Pharmacol. 106, 295-310.
23. SATO, A. AND NAKAJIMA, T. (1979) Partition coefficients of some aromatic hydrocarbons and ketones in water, blood and oil. Br. J. Industr. Med. 36, 231-234.
24. GARGAS, M.L., BURGESS, R.J., VOISARD, D.E., CASON, G.H. AND ANDERSEN, M.E. (1989) Partition coefficients of low-molecular-weight volatile chemicals in various liquids and tissues. Toxicol. Appl. Pharmacol. 98, 87-99.
25. PEETERS, L.L.H., GRUTTERS, G. AND MARTIN, JR., C.B. (1980) Distribution of cardiac output in the unstressed pregnant guinea pig. Am. J. Obstet. Gynecol. 138, 1177-1184.
26. BRYANT, P.J.R., FORD-MOORE, A.H., PERRY, B.J., WARDROP, A.W.H. AND WATKINS, T.F. (1960) Preparation and physical properties of isopropyl methylphosphonofluoridate. J. Chem. Soc., 1553-1555.
27. BENSCHOP, H.P., BIJLEVELD, E.C., OTTO, M.F., DEGENHARDT, C.E.A.M., VAN HELDEN, H.P.M. AND DE JONG, L.P.A. (1985) Stabilization and gas chromatographic analysis of the four stereoisomers of 1,2,2-trimethylpropyl methylphosphonofluoridate (soman) in rat blood. Anal. Biochem. 151, 242-253.
28. BENSCHOP, H.P., KONINGS, C.A.G., VAN GENDEREN, J. AND DE JONG, L.P.A. (1984) Isolation, anticholinesterase properties, and acute

toxicity in mice of the four stereoisomers of the nerve agent soman. Toxicol. Appl. Pharmacol. 72, 61-74.

29. HARRIS, L.W., BRASWELL, L.M., FLEISHER, J.H. AND CLIFF, W.J. (1964) Metabolites of pinacolyl methylphosphonofluoridate (soman) after enzymatic hydrolysis in vitro. Biochem. Pharmacol. 13, 1129-1136.
30. FLEISHER, J.H. AND HARRIS, L.W. (1965) Dealkylation as a mechanism for ageing of cholinesterase after poisoning with pinacolyl methylphosphonofluoridate. Biochem. Pharmacol. 14, 641-650.
31. DEPARTMENTS OF THE ARMY AND THE AIR FORCE (1975) Military chemistry and chemical compounds. Field manual, FM 3-9/AFR 355-7, Washington, DC, p. 3-4.
32. KEIJER, J.H. AND WOLRING, G.Z. (1969) Stereospecific aging of phosphonylated cholinesterases. Biochim. Biophys. Acta 185, 465-468.
33. OOMS, A.J.J. AND BREEBAART-HANSEN, J.C.A.E. (1965) The reaction of organophosphorus compounds with hydrolytic enzymes. The inhibition of horse liver aliesterase. Biochem. Pharmacol. 14, 1727-1738.

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BIBLIOGRAPHY OF PUBLICATIONS AND MEETING ABSTRACTS

LANGENBERG, J.P., VAN DIJK, C., DE JONG, L.P.A., SWEENEY, R.E. AND MAXWELL, D.M. (1993) Physiologically based model for the toxicokinetics of C(±)P(±)-soman in the guinea pig. Proceedings of 1993 Medical Defense Bioscience Review, Baltimore, MD, 10-13 May 1993, pp. 675-684.